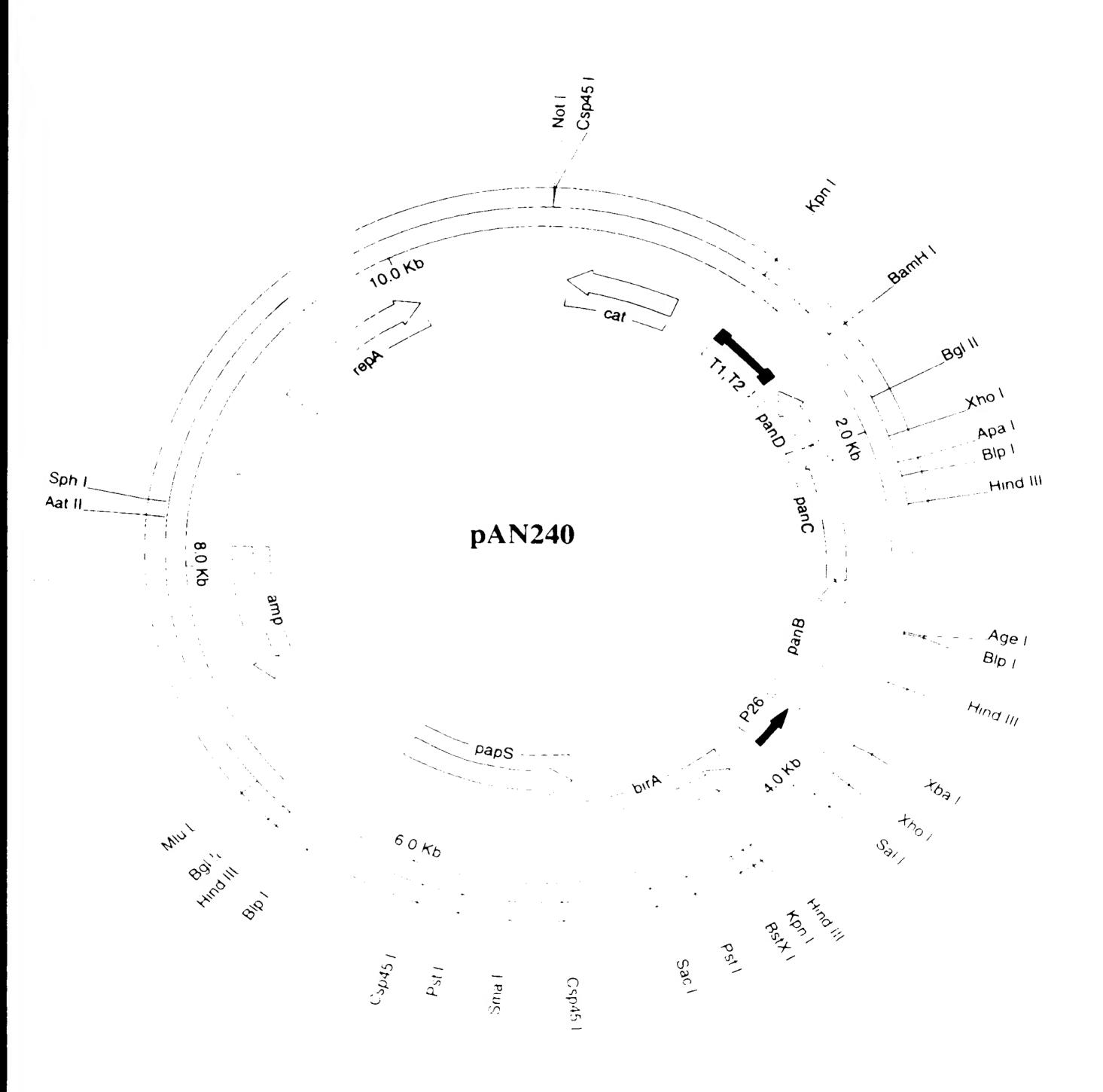
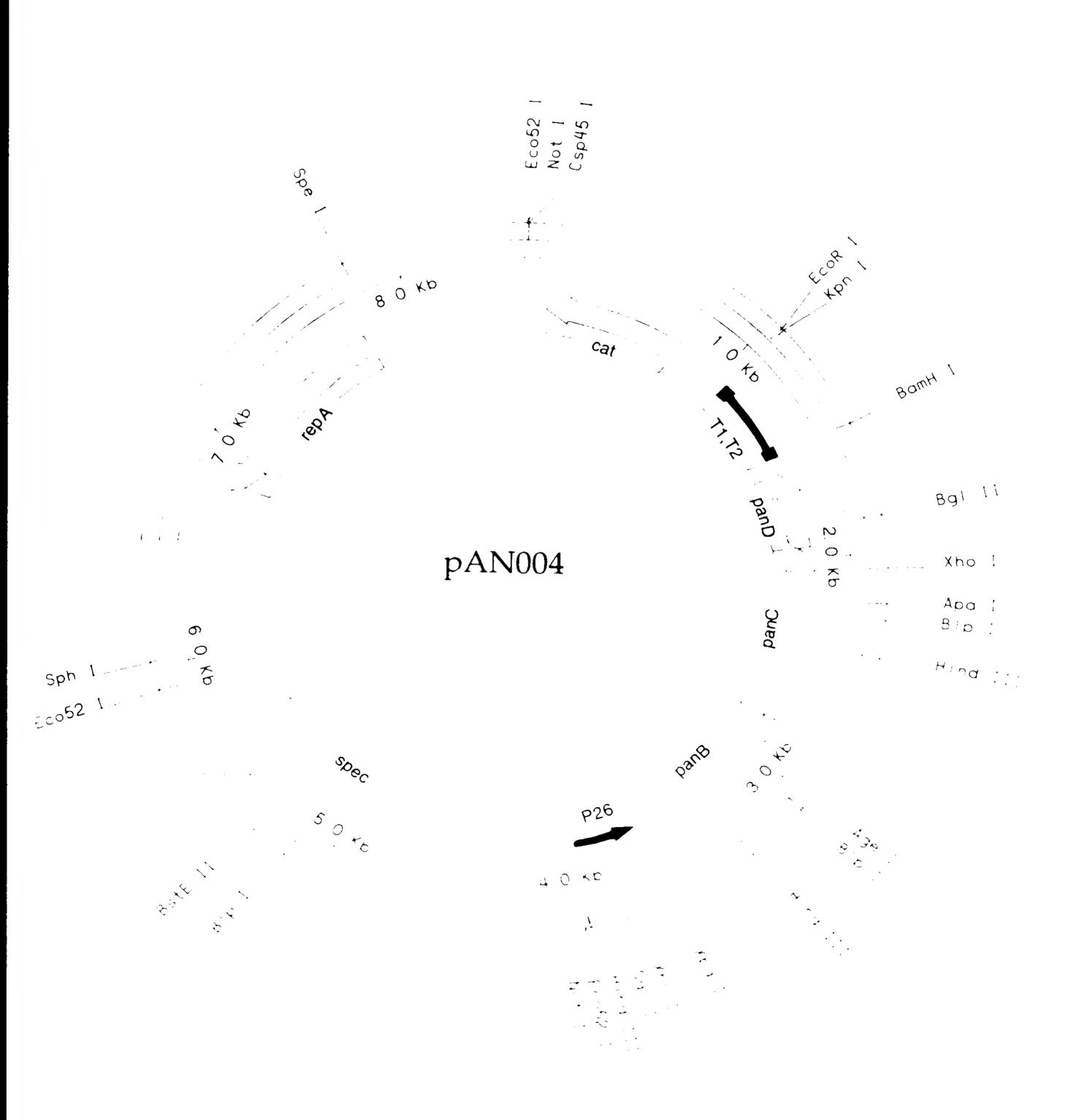


Figure 2. Plasmid pAN240, containing sequences ligated upstream of the P<sub>26</sub>panBCD cassette, equivalent to the integrated version in strain PA221.





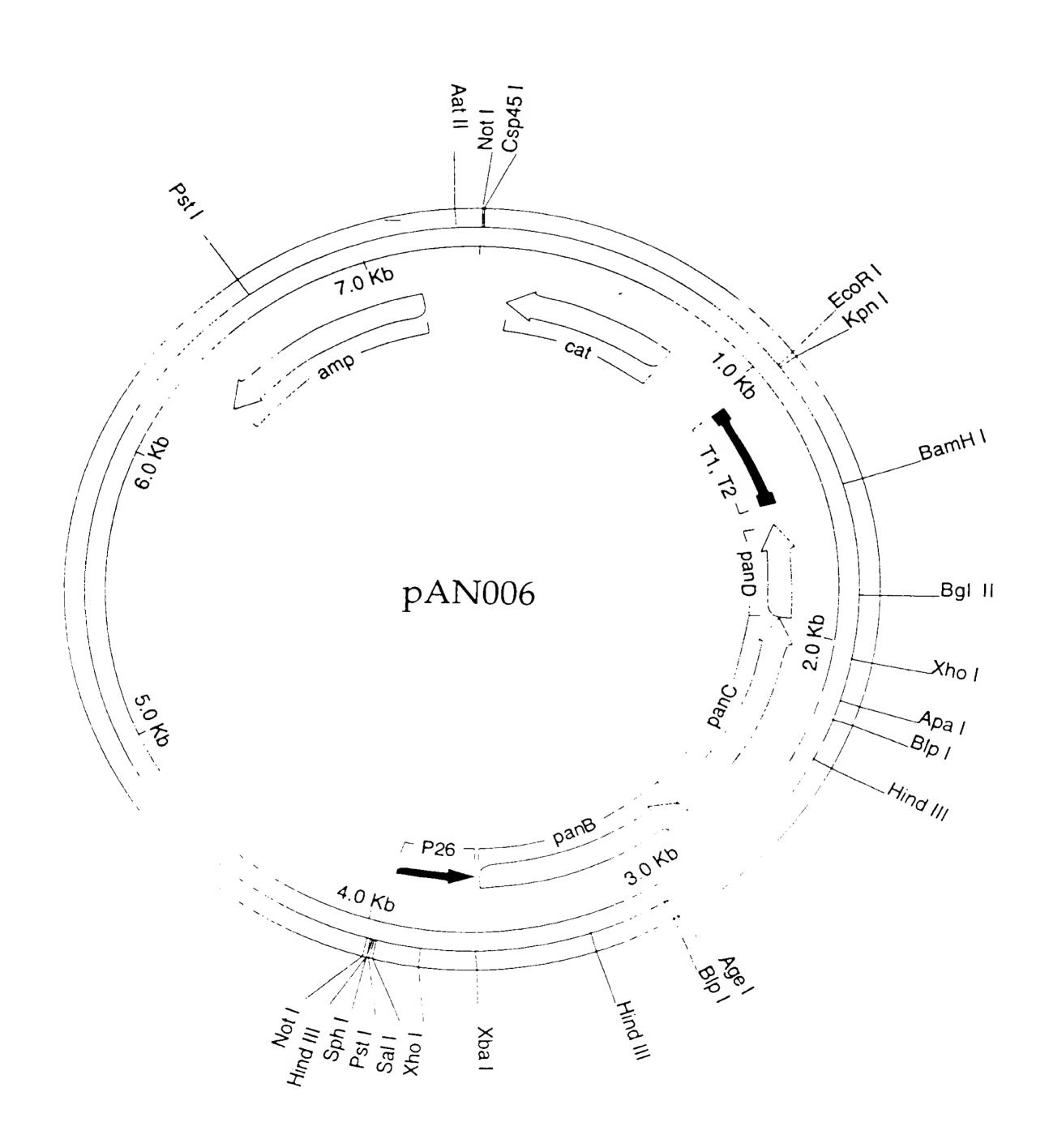
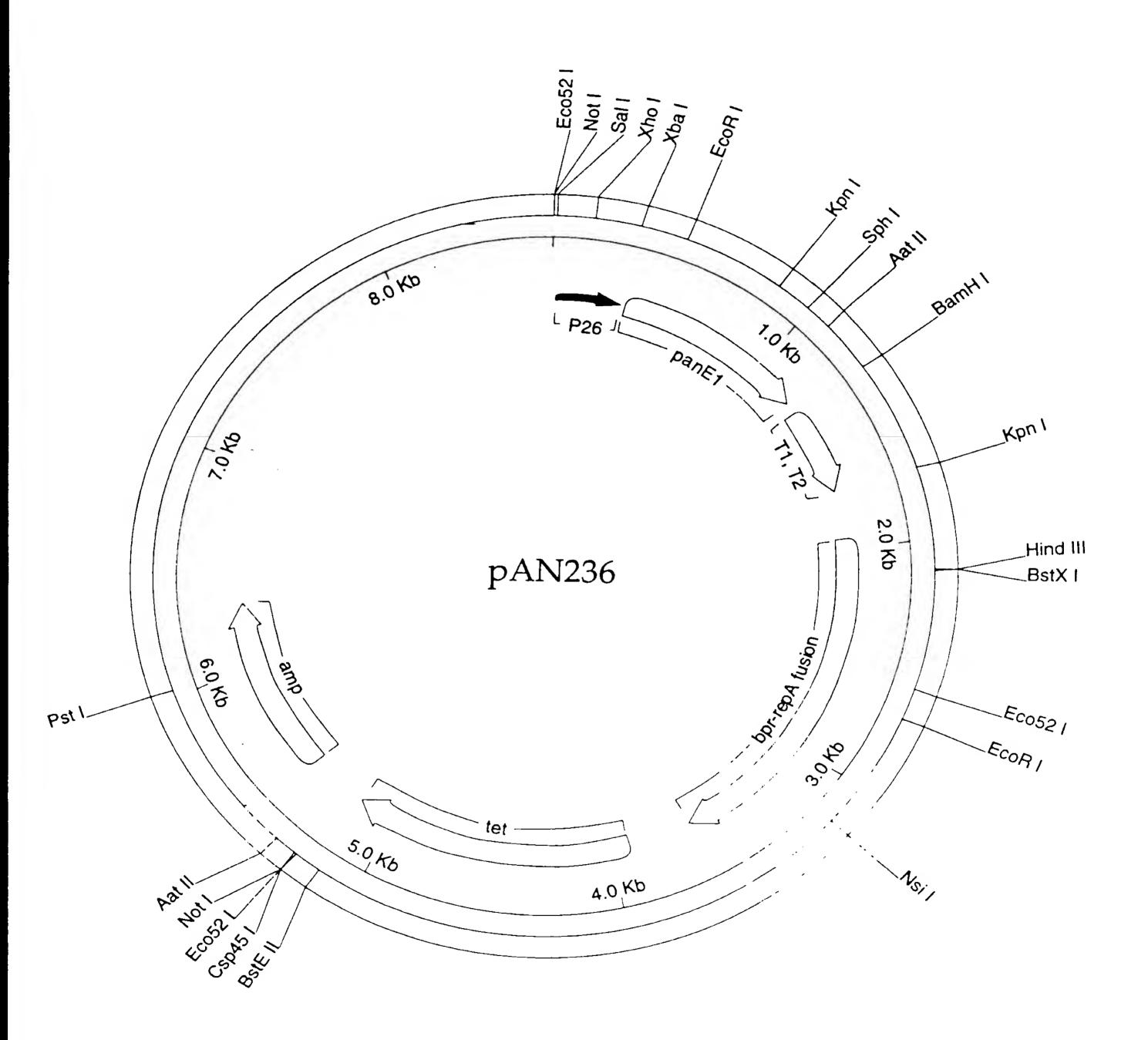
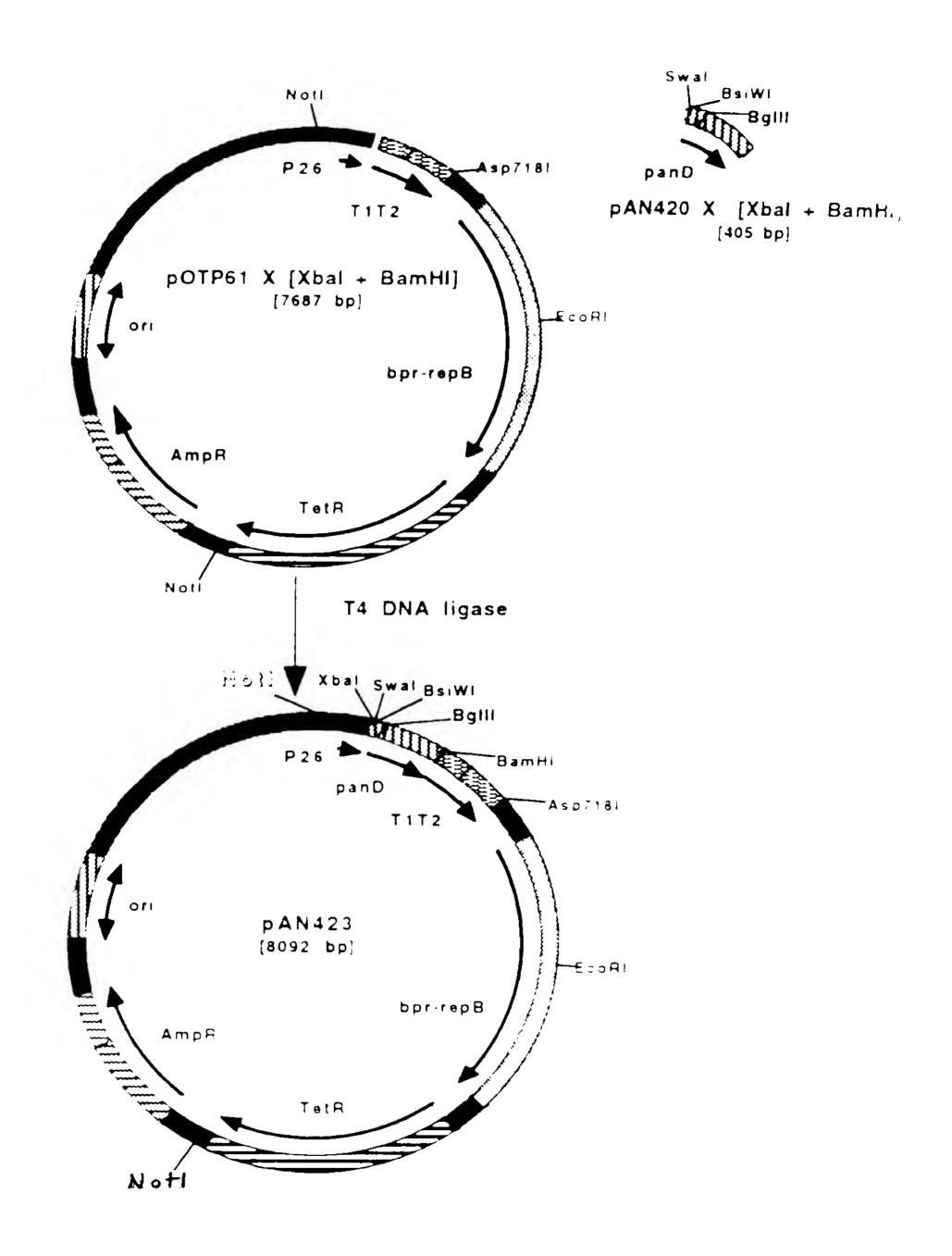
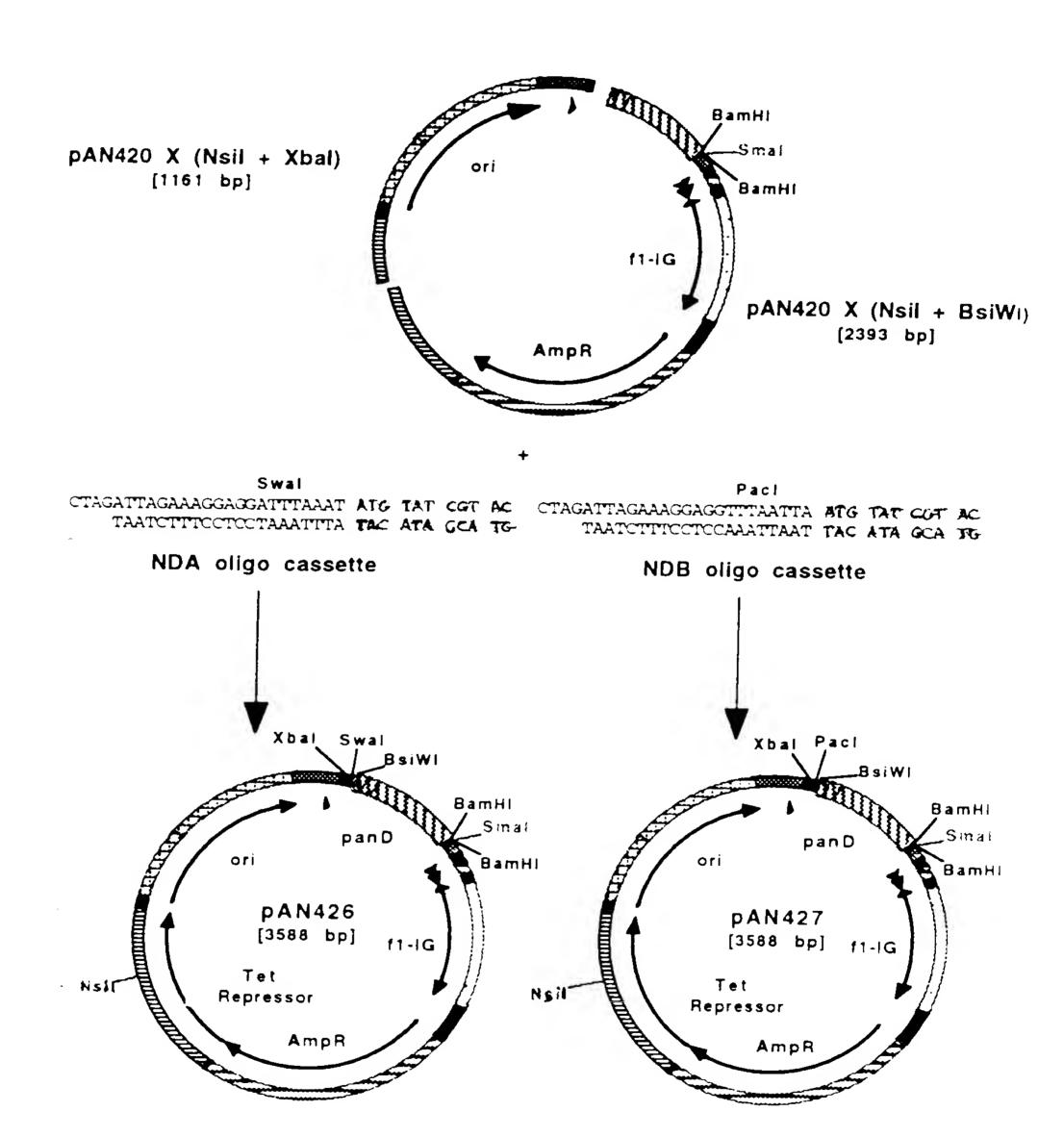
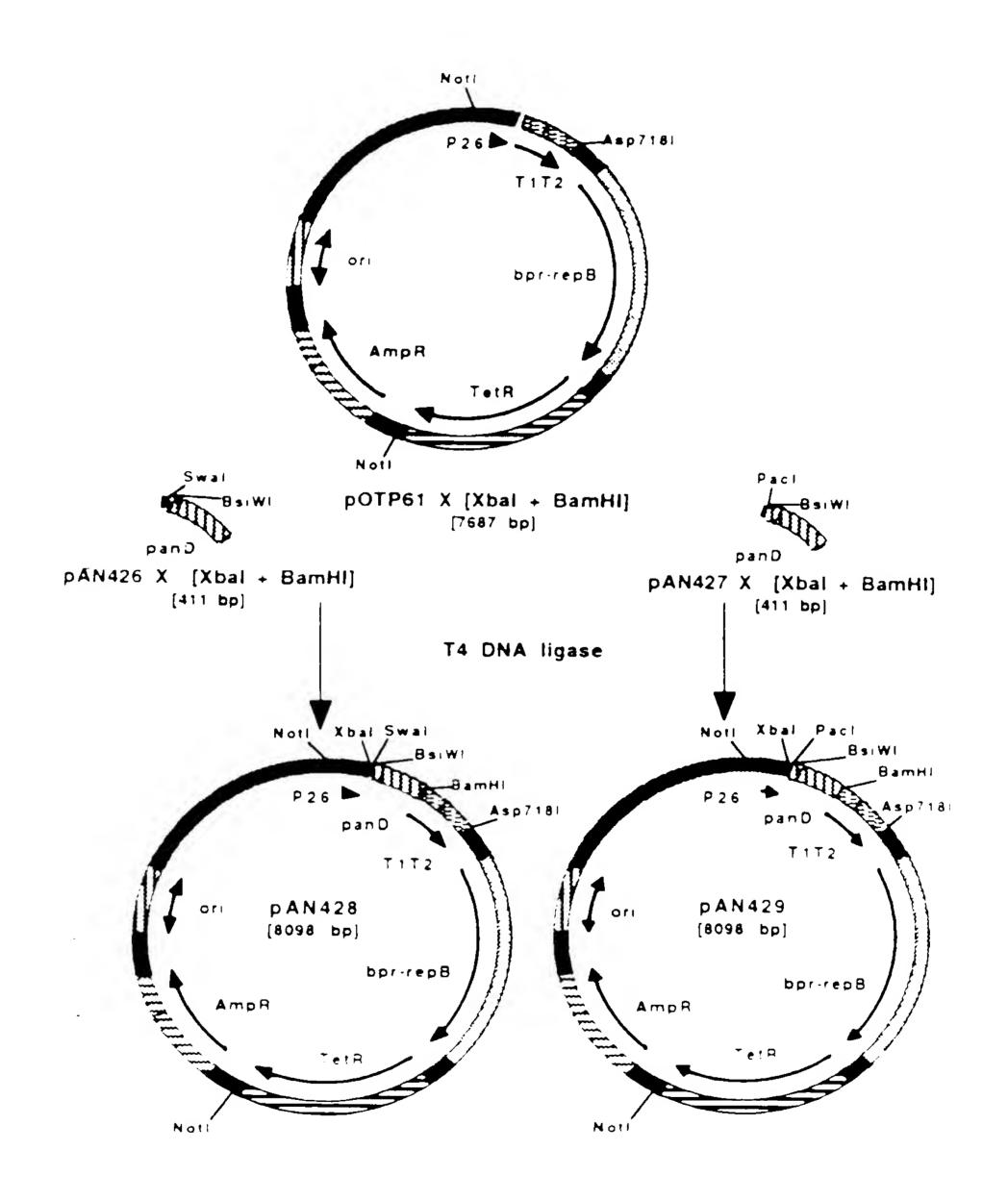


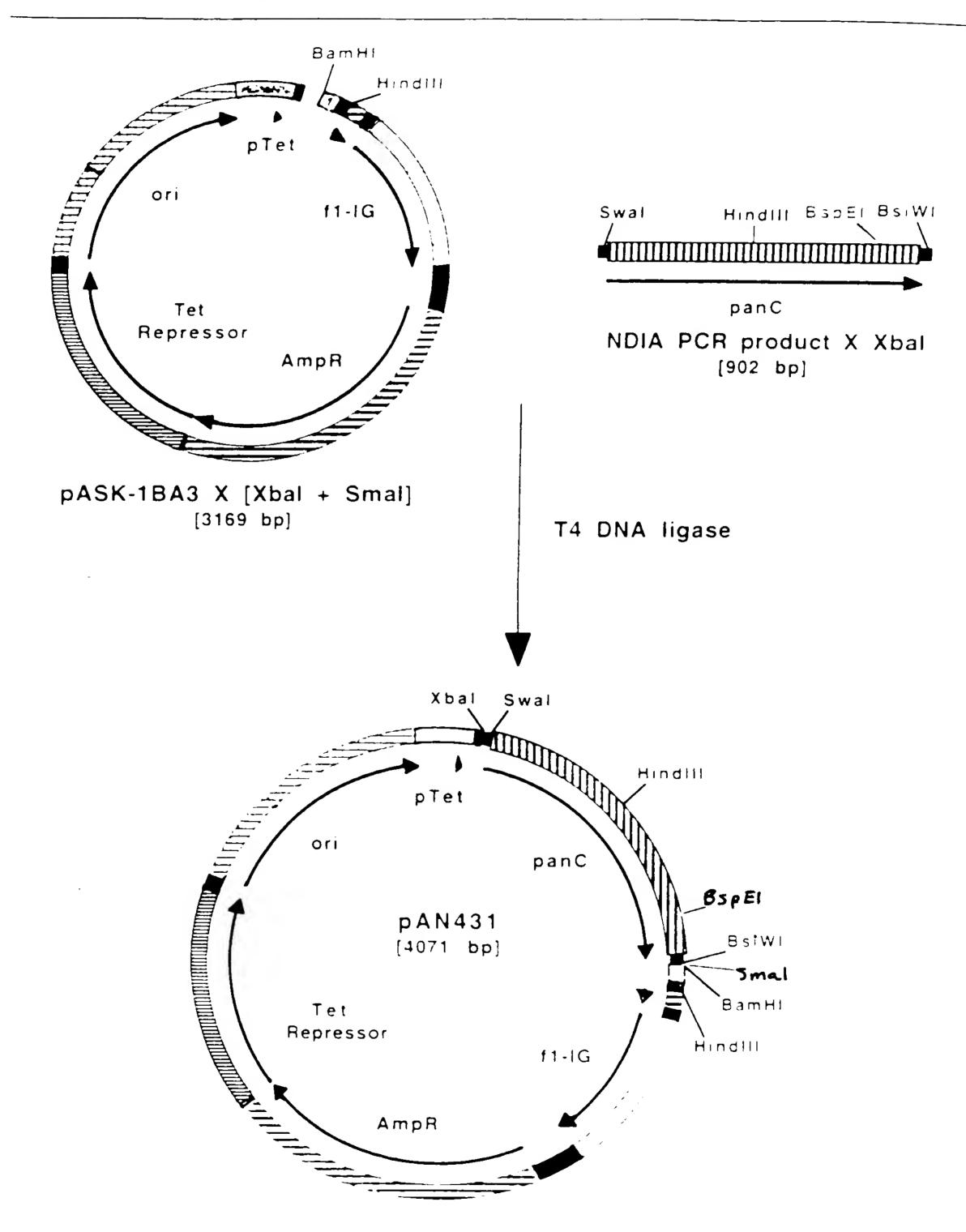
Figure 4 Plasmid pAN236, containing an integratable and amplifiable P26-RBS2-panE1 expression cassette.

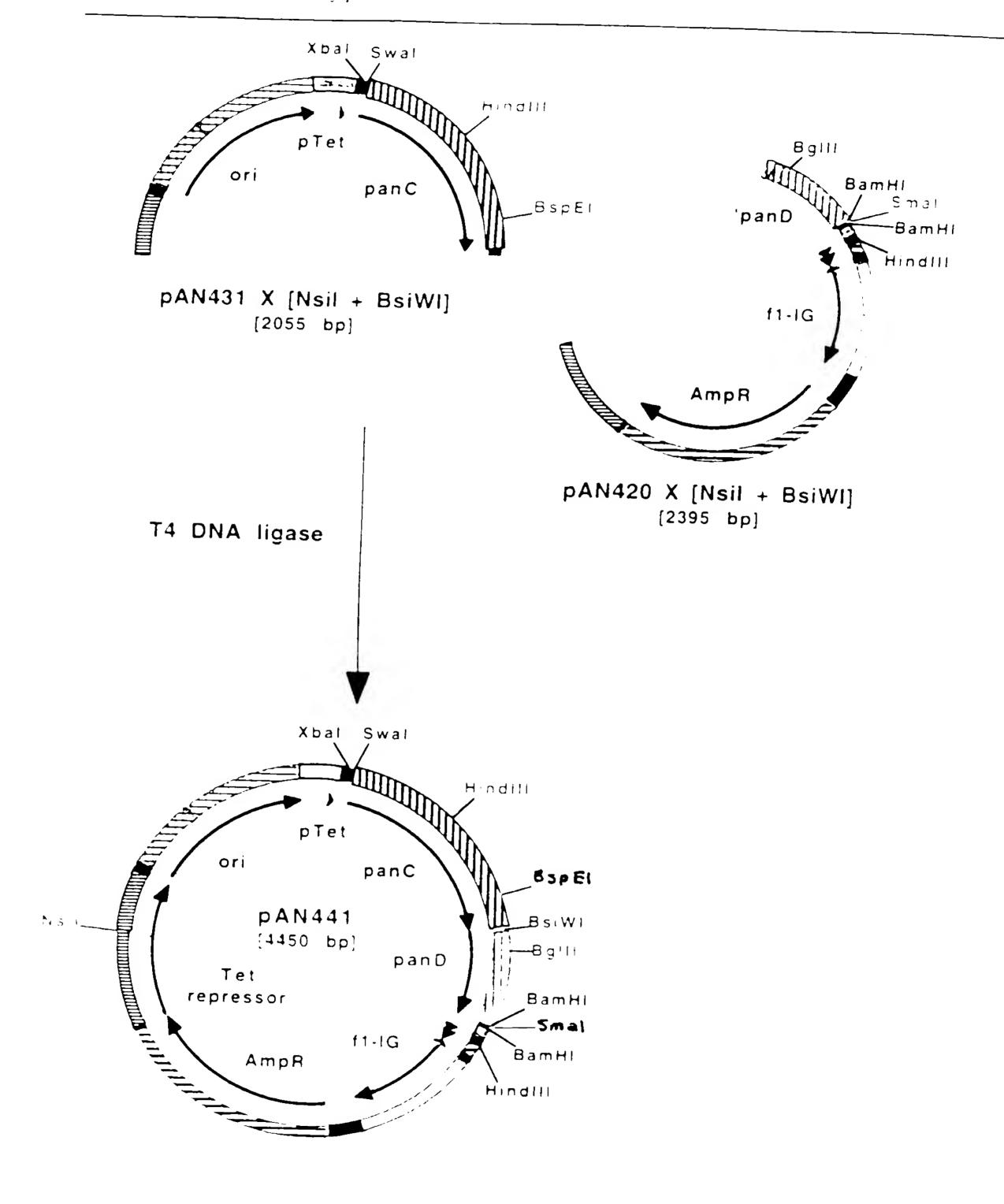












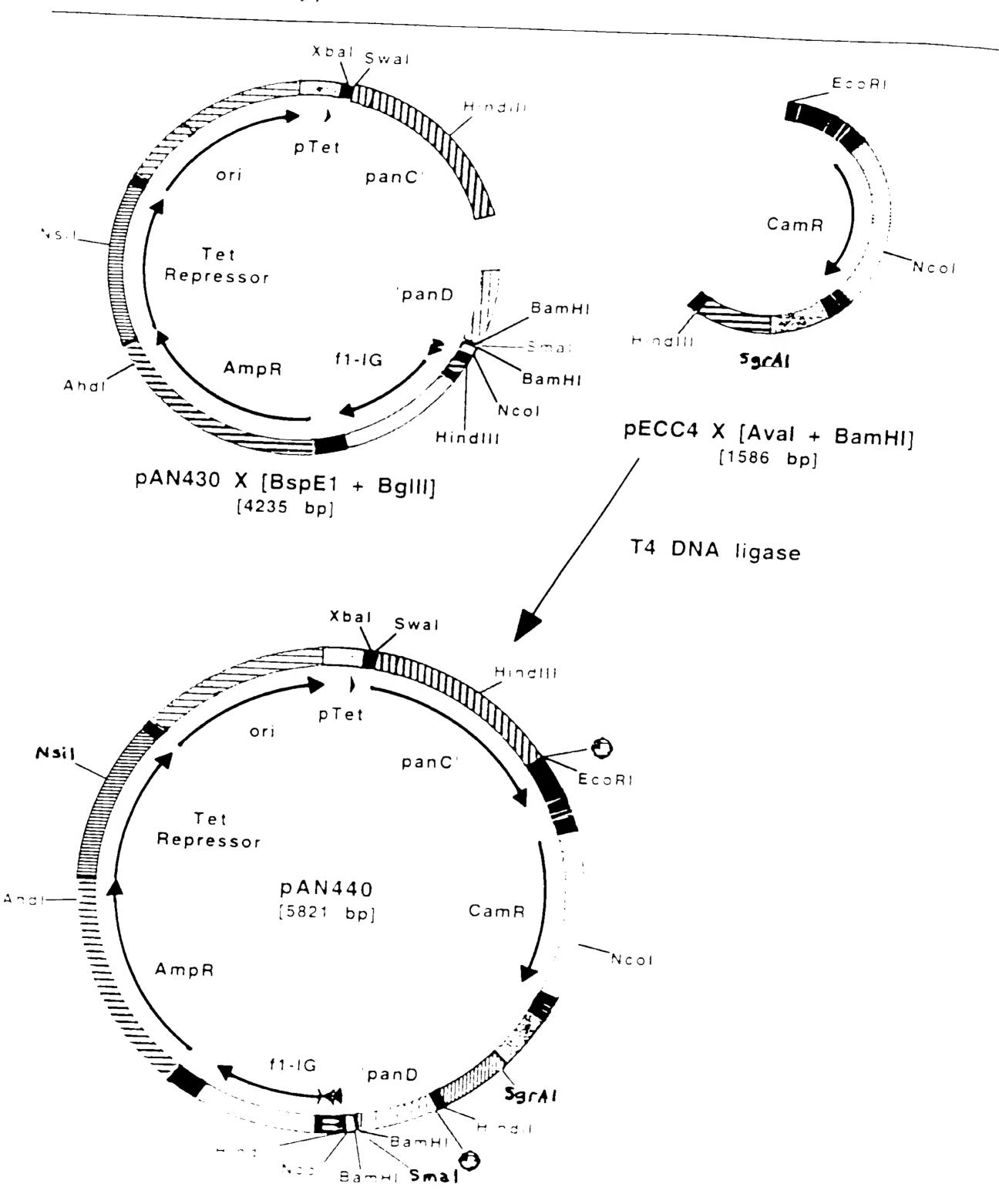


Figure || Structure of pAN251, a plasmid designed to integrate a single copy of P<sub>26</sub> panE1 at the panE1 locus by double crossover.

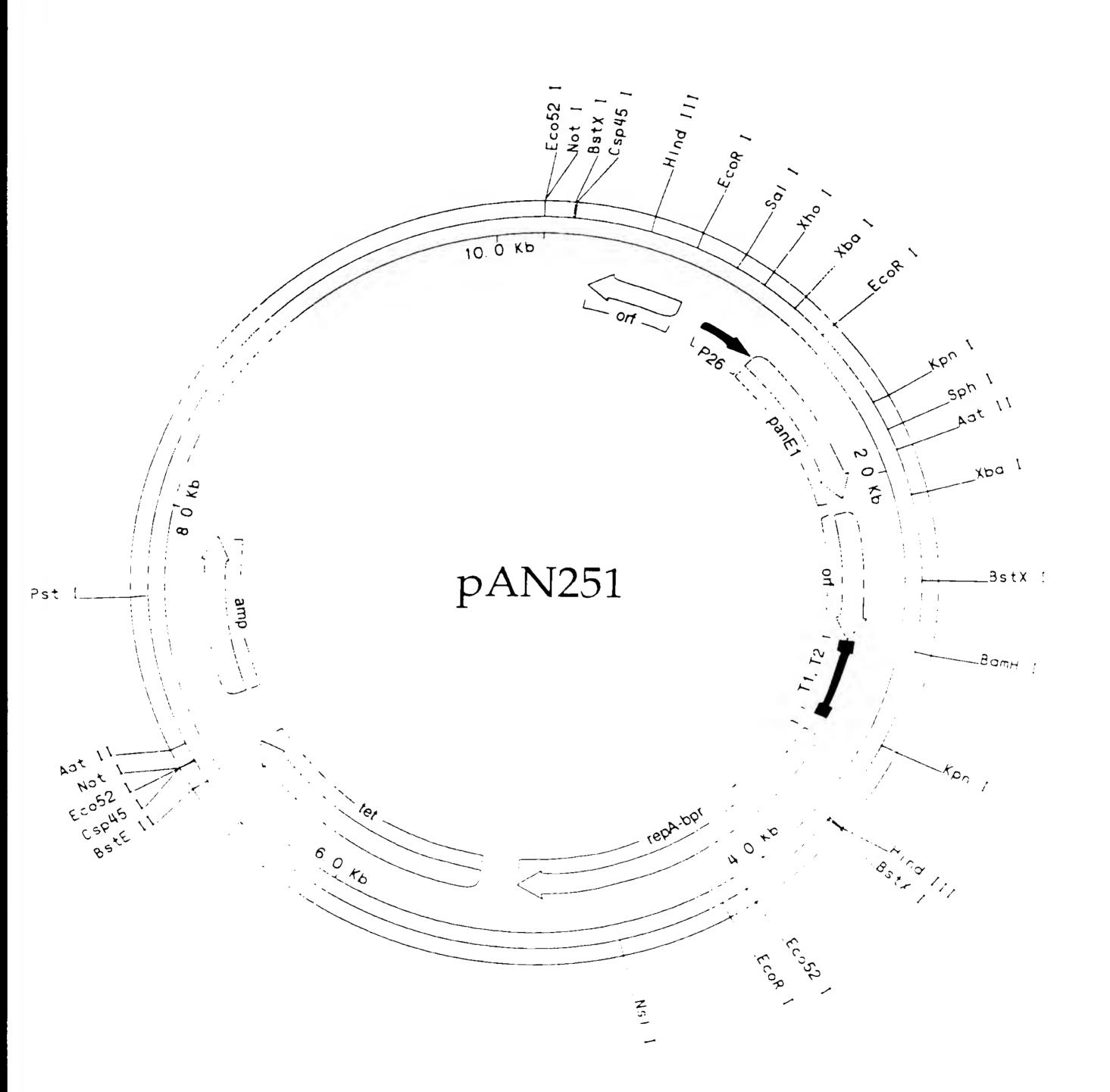


Figure 12 Structure of pAN267, a plasmid designed to stably integrate a P<sub>26</sub> ilvBNC cassette at the amyE locus.

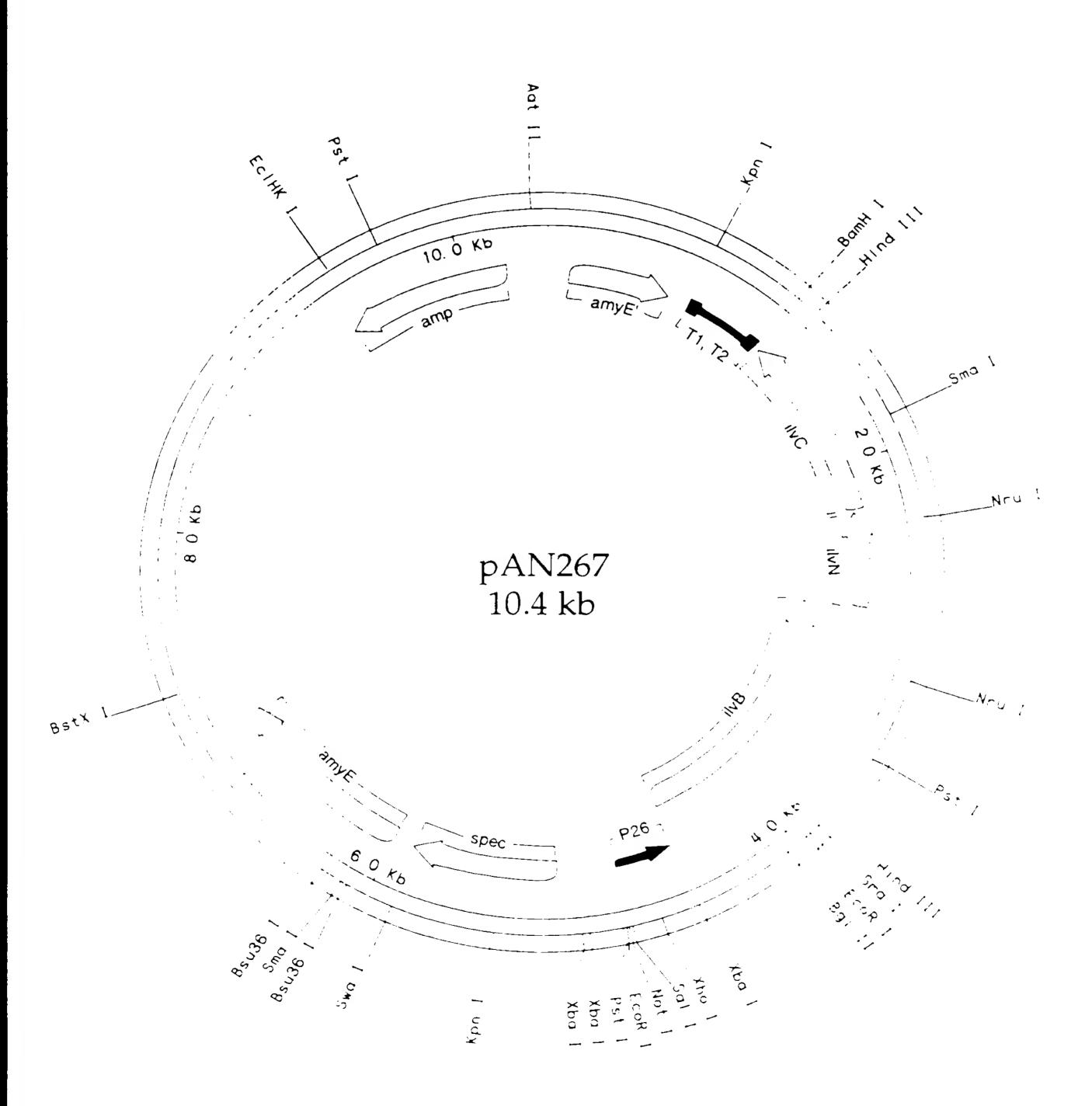


Figure 13 Structure of pAN257, a clone of B. subtilis ilvD in a low copy vector.

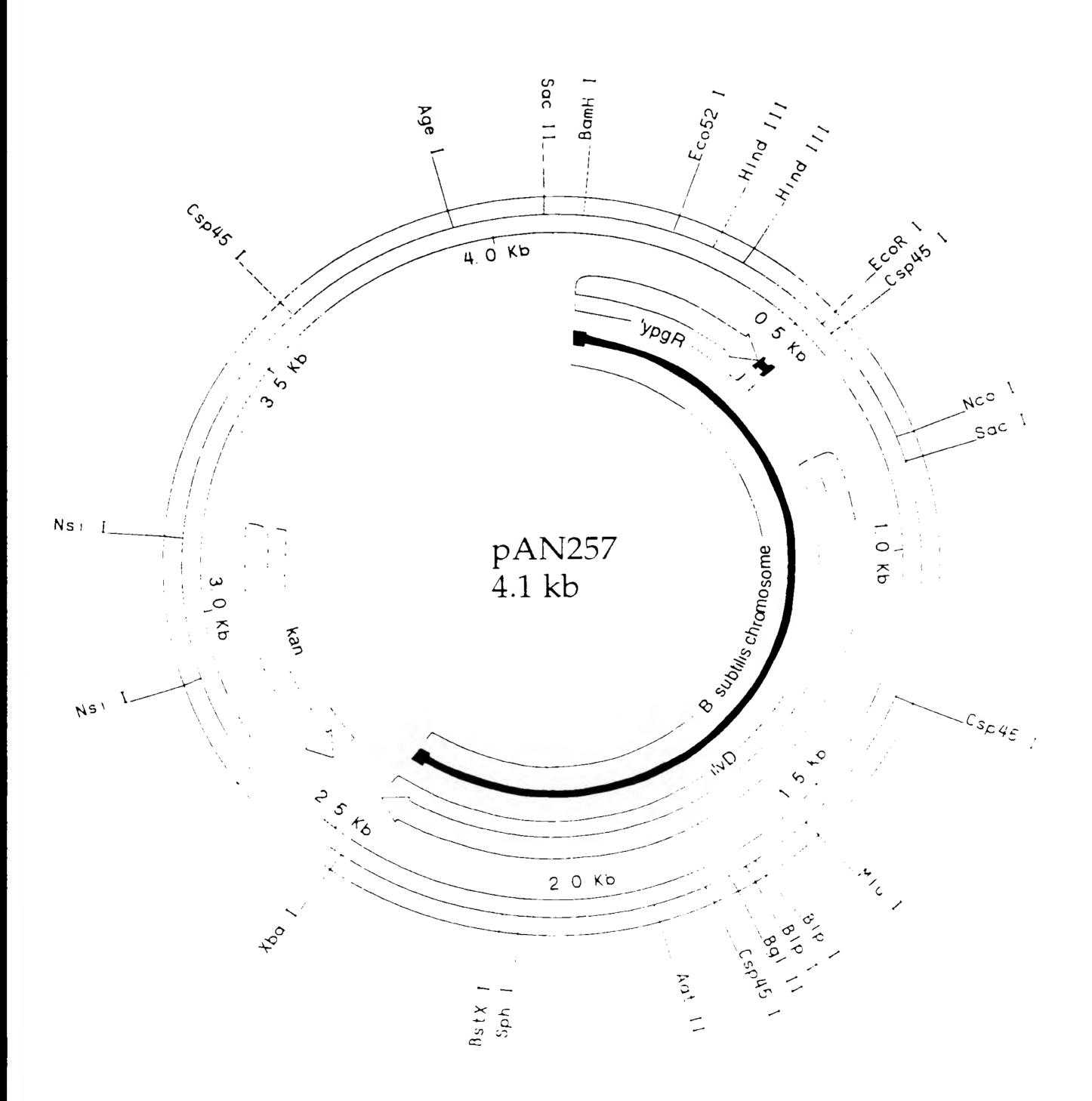


Figure 14 Structure of pAN263, designed to stably integrate a single copy of  $P_{26}$  ilvD at the ilvD locus.

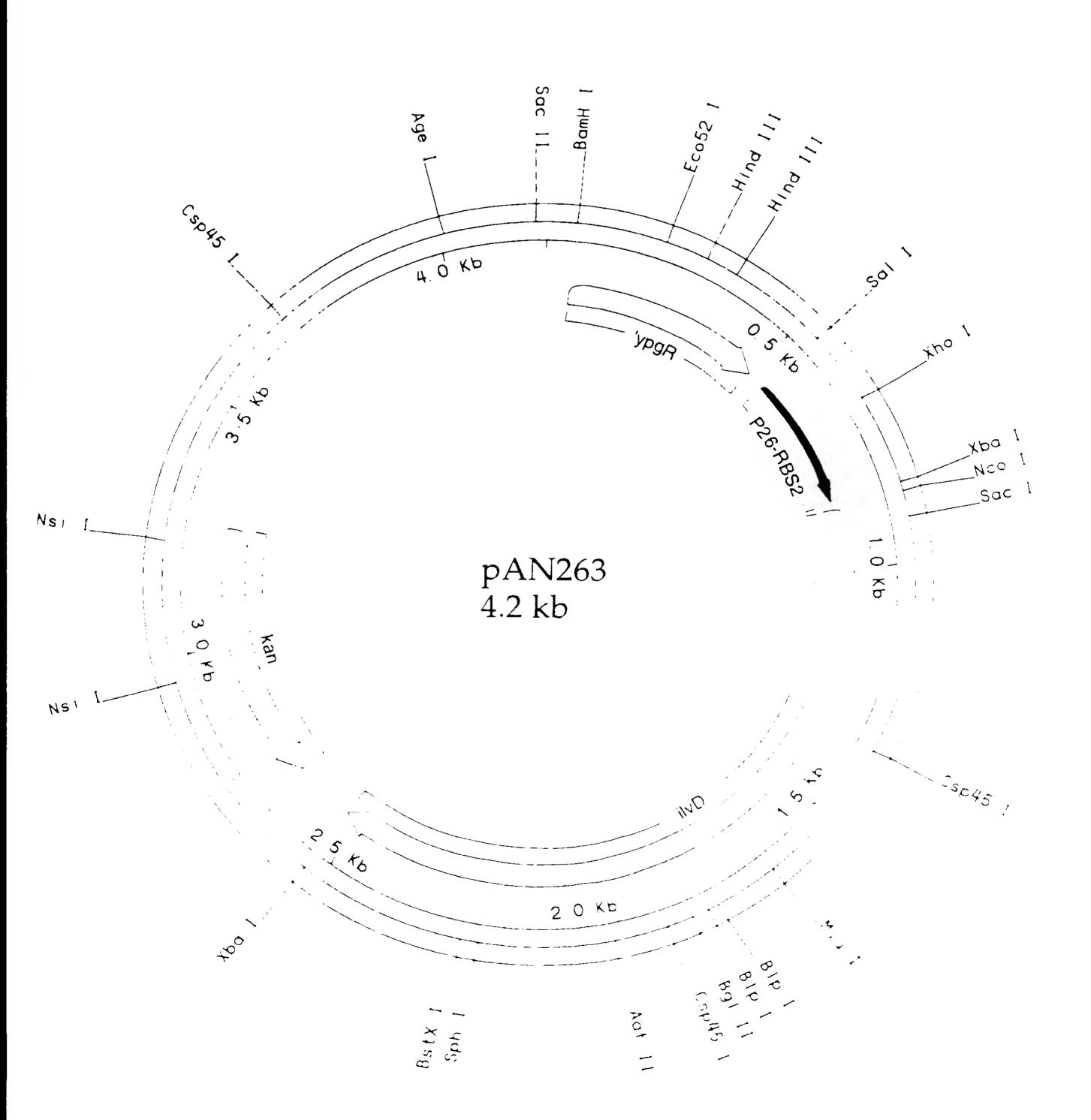
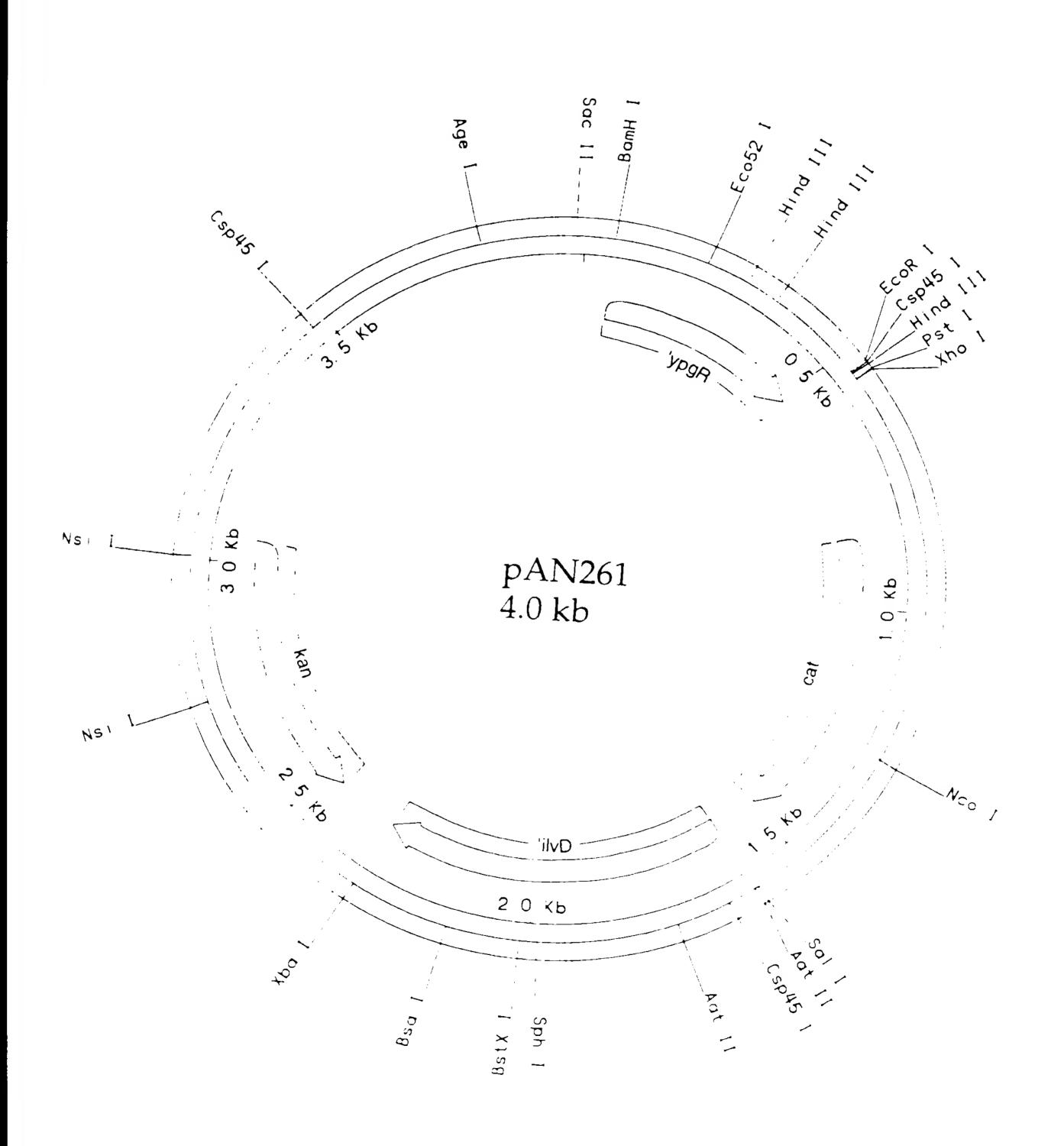


Figure 15 Structure of pAN261, designed to disrupt the B. subtilis ilvD gene with the cat gene.



#### pantothenate **ATP** pantothenate kinase (coaA, panK, coaX) ADP **←** 4'-phosphopantothenate CTP, cysteine 4'-phosphopantothenylcysteine 4'-phosphopantetheine **ATP** coaD Dephosphocoenzyme A ATP ADP Coenzyme A apo-ACP

Holo-ACP

Figure 17 Structure of pAN296, designed to delete most of the B. subtilis coaA gene and substitute a chloramphenicol resistance gene.

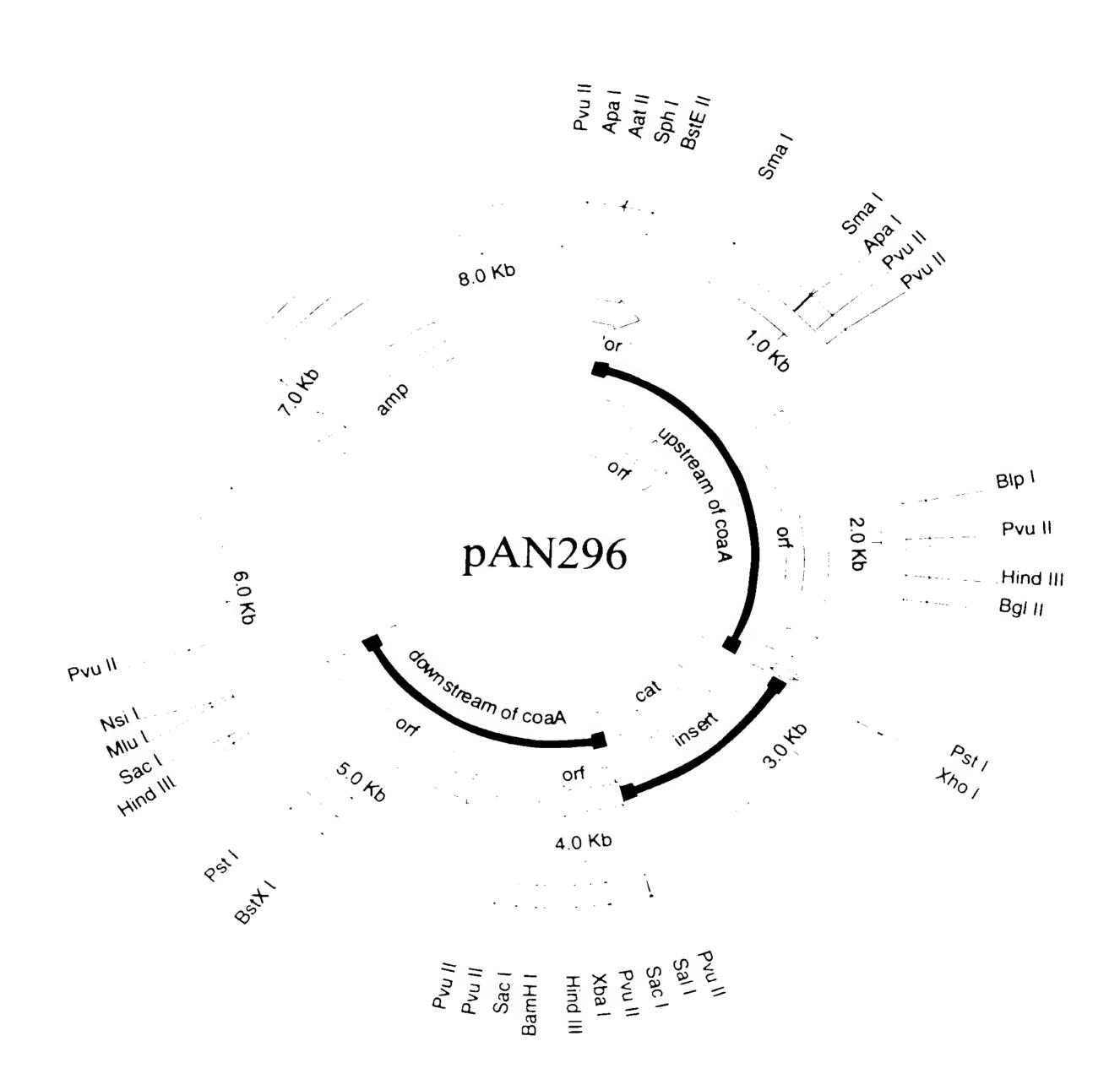


Figure 18 Structure of the B. subtilis chromosome in the region of the coad gene. The scale is in base pairs and the significant open reading frames are shown by the open arrows.

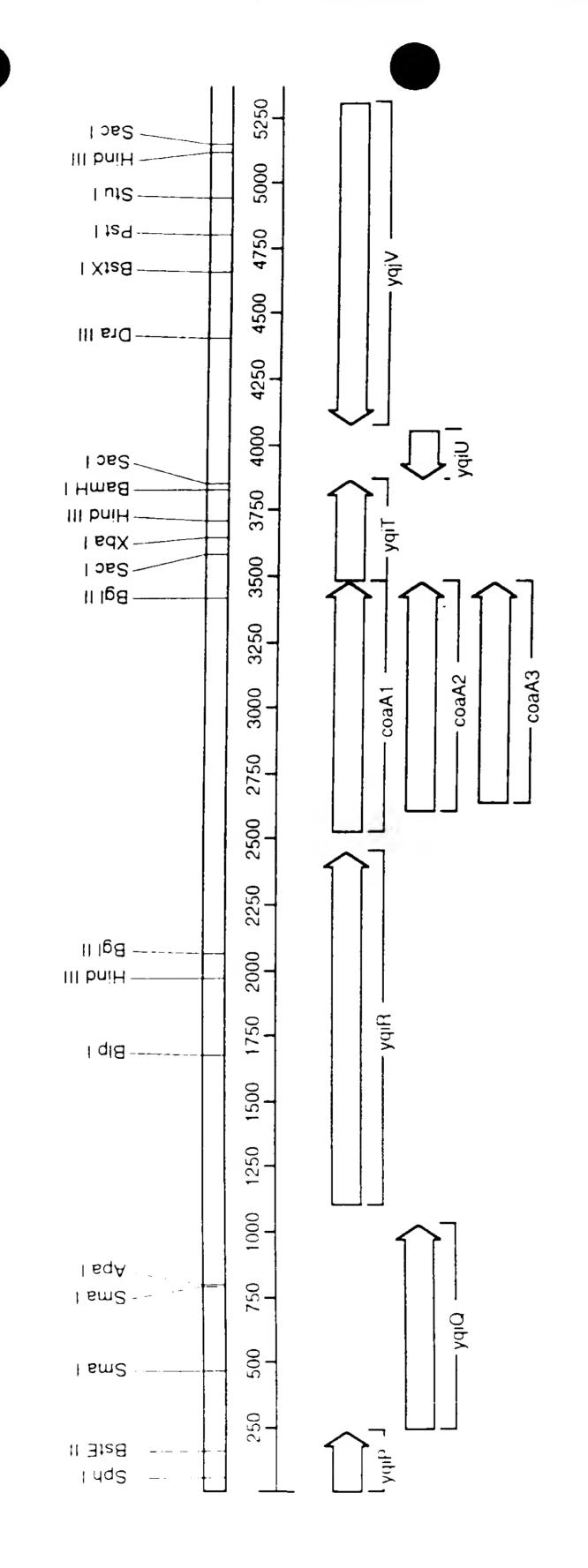
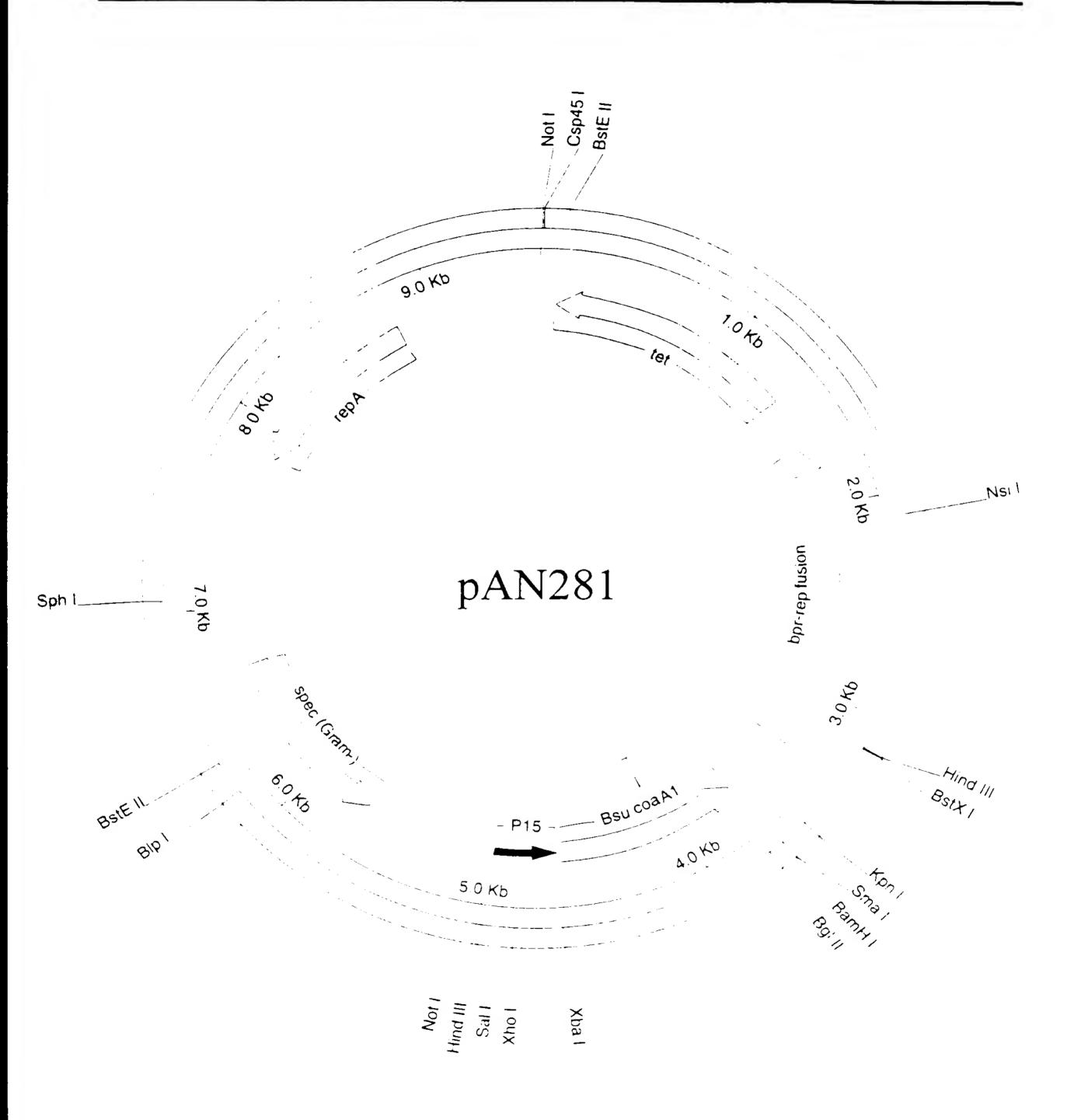


Figure 19 Structure of p.4N281, a plasmid for expressing B. subtilis coaA after integration at the bpr locus. pAN282 and pAN283 have similar structures.



## FIG.20A

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# FIG.20B

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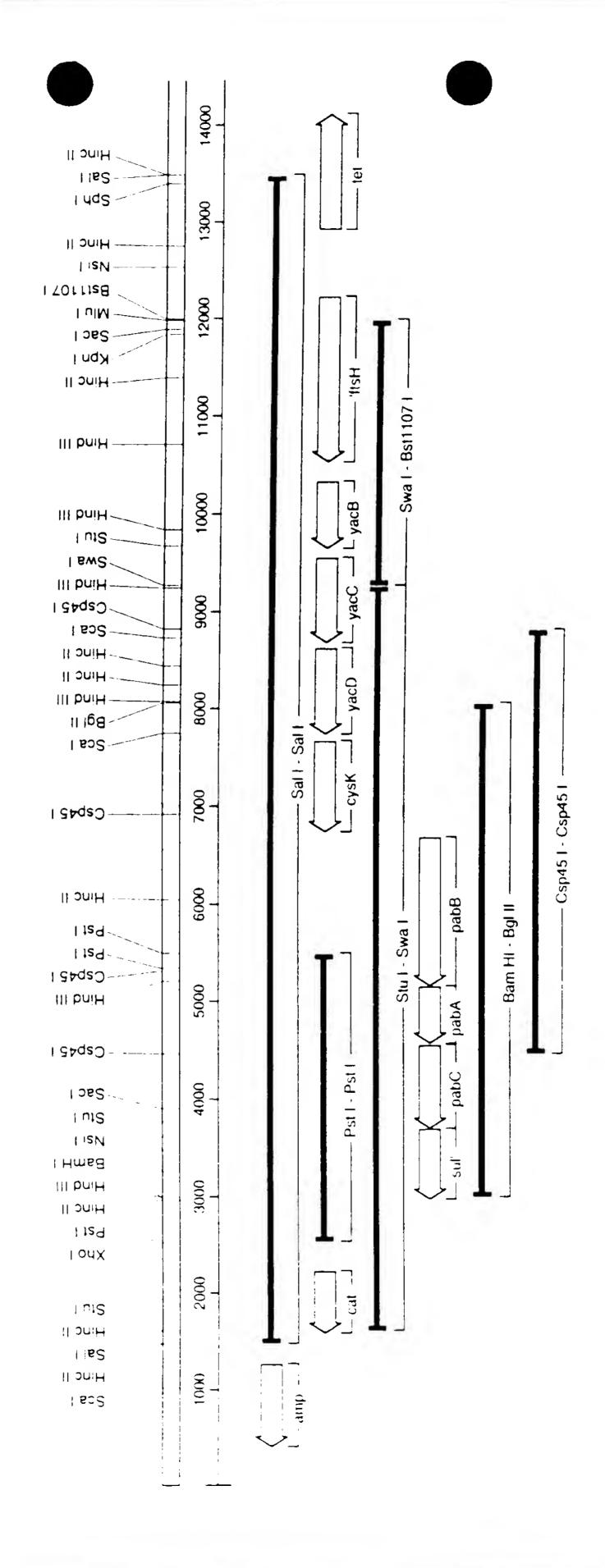
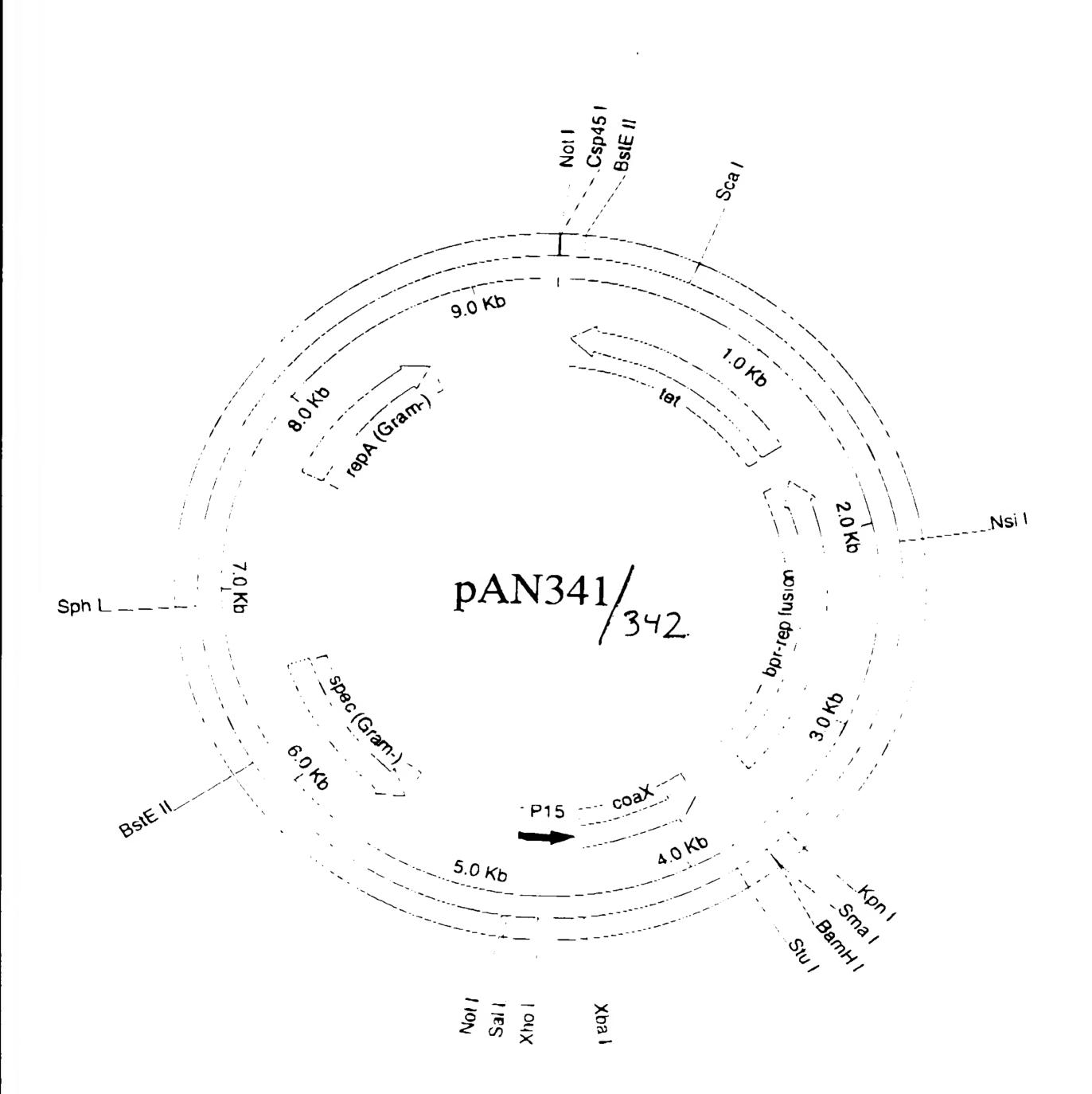


Figure 22 Structure of pAN341 and pAN342, two independent PCR- derived clones of yacB (renamed coaX).



## **FIG.23A**

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#### **FIG.23C**

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GRA--CITTLE POTATTET TRATISTES TRATISTES TERMANDER

GRA--CITTLE POTATTET TRATISTES TRATISTES TERMANDER

KN+-GITTLE POTATTET TRATISTES TRATISTES TRATISTES

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# FIG.23D

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Figure 24 Alignment of a portion of the amino acid sequences of several known or suspected pantothenate kinases. The residues that are mutated in E. coli coa A15(Ts) and B. subtilis coa A from plasmid pAN282A are indicated below and above the alignment, respectively. The coordinate given in the left margin for the B. subtilis protein refers to the coad 1 open reading frame.

Majority		B. subtilis CoaA1	E. coli CoaA	H. influenzae CoaA	M. leprae CoaA	M. Inberculosis CoaA	S. coelecolor CoaA	
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Figure 25 Structure of p.AN294, a plasmid for integrating mutagenized B. subtilis coaA at its native locus.

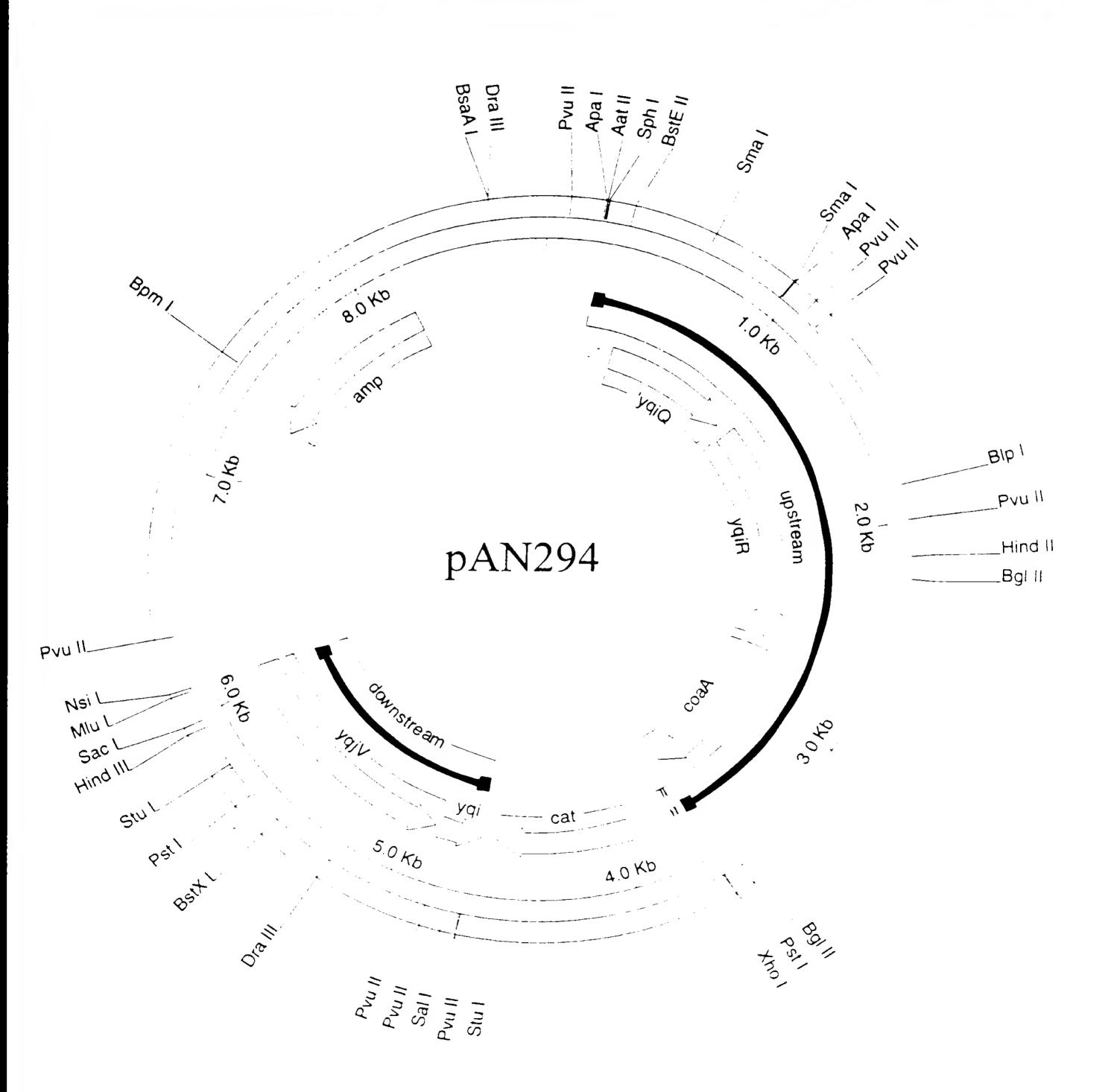


Figure 26 Structure of pAN336, a plasmid designed to delete B. subtilis coaX from the chromosome and replace it with a kanamycin resistance gene.

